

BVD testing for calves



Bovine Viral Diarrhoea (BVD) is an infectious disease of cattle caused by the bovine viral diarrhoea virus (BVDV).

Persistently infected (PI) animals are born with BVD and will excrete large amounts of the virus their whole life. These animals need to be identified to eliminate BVD from your herd.

Transiently infected (TI) cattle have been exposed to the virus through direct contact with other infected animals. They create antibodies to clear the virus over 2-3 weeks. These antibodies then protect the animal from future infection.

Tissue Sample Units (TSU) as provided by Allflex for collecting a tissue sample from the ear. This can contain a liquid preservative (wet TSU- calves over 35 days) or no preservative (dry TSU- Calves under 35 days).

How do you test animals for BVD if they are under 35 days old?

Collect a tissue sample using a dry TSU or a blood sample, send it to us, and we will test for the presence of BVD virus using PCR. The turnaround time and price for both tissue and blood PCR is a 6 days at \$14.40 per sample.

Why don't you test calves under 35 days old with the BVD Antigen ELISA?

Protective BVD antibodies are transferred from cows to calves via colostrum (maternal antibodies). Calves could have high levels of maternal antibodies circulating in their body for days to several weeks if they received pooled colostrum or colostrum from their dam. The job of these antibodies is to bind to the BVD virus, which can interfere with the Antigen ELISA process creating a false negative result. A false negative result means that a PI animal may be missed.

An LIC research trial in 2011 which confirms this:

A total of 11 PI calves were sampled (blood serum and tissue) at least 8 times between 0 and 56 days of age, with most sampling points occurring in the first 8 days of age. Some calves had colostrum intake on day 0 and 2, others on day 2 only. PCR testing of the serum gave positive BVD results at all sampling points for all 11 calves. However, the ability of the antigen ELISA test to detect BVD was poor within the first 8 days, with tissue samples showing either negative or suspect results on at least one occasion for each PI calf (Figure 1). Serum samples gave similar results for all 11 calves (data not shown). Of concern, there were 3 PI calves that gave negative or suspect results until they were 8, 14 or 28 days of age, which was evident with both tissue and serum samples (example of one PI calf in Figure 2). Due to this diagnostic gap, LIC has always applied the rule that calves must be over 35 days of age before using antigen ELISA.

	Age of calf (d)										
	0	1	2	4	6	8	11-16	17-22	24-29	31-56	
PI-1	NEG	NEG	NEG	NEG	SUS						
PI-2	NEG	NEG	NEG								
PI-3	NEG	NEG	NEG								
PI-4	NEG	NEG	SUS	NEG							
PI-5	NEG	NEG	NEG								
PI-6	NEG	NEG	NEG	SUS	SUS						
PI-7	SUS	NEG	NEG	NEG	NEG	NEG					
PI-8	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG		
PI-9	NEG	NEG	NEG	NEG	NEG	NEG	NEG	NEG			
PI-10	NEG	NEG	NEG	SUS							
PI-11	NEG	NEG									

No test	Non PI	TI	PI
	Negative	Suspect	Positive

Figure 1. Tissue antigen ELISA results for 11 PI calves tested between 0 and 56 days of age. Results assigned according to kit manufacturer's instructions.

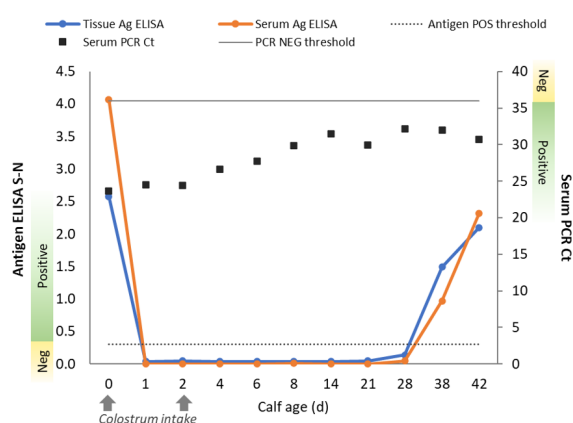


Figure 2. Tissue and serum antigen ELISA sample - negative ratio (S-N) and serum PCR cycles (Ct) for one PI calf sampled between 0 and 42 days of age. Results are shown relative to the test thresholds applied for ELISA and PCR. Colostrum intake occurred at days 0 and 2.

Why do maternal antibodies from the cow not affect BVD tissue PCR?

PCR directly detects unique genetic material (RNA) from the BVD virus. The presence of any maternal antibodies has no effect on this detection. In contrast, Antigen ELISA methodology uses a special antibody-based capture system to detect proteins on the surface of the virus, which is why the maternal antibodies present in the calf can interfere with this test and cause false negative results.

Can you distinguish between PI and TI animals using PCR?

The results from our validation trials have allowed us to set PCR test thresholds which distinguish between animals that are likely to be a PI versus TI. The categories for BVD tissue PCR include the following, and are included on all results reports you receive: High Positive: Most likely a PI, you may choose to cull based on a single test result. Alternatively, you may choose to confirm PI status by collecting and testing another sample 4 weeks later. Positive: Possibly a PI, but occasionally transient infections may give a Positive result. To confirm PI status, we recommend a second sample is collected and tested 4 weeks later. Weak Positive: Low levels of BVD virus detected. The animal may be a PI but likely transiently infected and recovering. Recommend PI status confirmation. Negative: A single, negative PCR result is sufficient to confirm that the calf is not a PI. If the calf becomes infected in future, it will rapidly develop immunity and rid itself of the virus. In pregnant cows however, acute infection may lead to her calf becoming a PI.

Why does LIC recommend confirmation testing?

An infection may be transient rather than persistent, particularly if the result is 'Positive' or 'Weak Positive' (i.e. not 'High Positive'). The only way to confirm this is by testing for the BVD virus again 4 weeks later. If the animal is still positive, they are a PI. If they now have a negative result, they were transiently infected and have cleared the virus.

In the 2020/21 season, less than 0.1% of calves tested by BVD tissue PCR were recommended for resampling and retesting ('Positive' or 'Weak Positive' result). Confirmation testing also mitigates the risk of incorrect labelling at first sample collection.

BVD serum PCR trial:

In a 2018 trial of mixed age animals, blood serum samples collected 4 weeks apart were tested by BVD PCR. All animals in the High Positive or Positive category at 1st test were PI. All those in the Weak Positive category at 1st test were later found to be negative, indicating that these were transiently infected animals.

1 st test result category		2 nd test result (4 weeks later)	
		Positive (PI)	Negative (Non-PI)
	High Positive, Positive	38	0
	Weak Positive	0	6
	Negative	0	22

BVD tissue PCR trial: Farm trial in 2019 compared the new BVD tissue PCR method against blood serum PCR as the gold standard, with excellent results.

Animals under 35 d of age	Tissue	Serum
High Positive, Positive, Weak Positive	18	18
Negative	466	466

Why do I have to use dry tissue sampling units if I want to do BVD tissue PCR?

The liquid preservative that is currently included in the Allflex wet TSU is not compatible with PCR test. The test will not work if the tissue has been in contact with this liquid. Allflex and LIC are currently investigating an alternative liquid preservative that is compatible with PCR.